CLAIMS

1. Monoclonal antibody which forms an immunological complex with a phosphorylated epitope of an antigen belonging to human abnormally phosphorylated tau protein, with said tau protein being liable to be obtained from a brain homogenate, itself isolated from the cerebral cortex obtained from a patient having Alzheimer's disease or having died of Alzheimer's disease.

2. Monoclonal antibody according to claim 1 characterized by the fact that it forms an immunological complex

- either with the peptide YSSPGSPGT or YSSPGSPGT, preferably YSSPGSPGT, phosphorylated at the positions marked with *
- or with any other peptide capable of forming an immunological complex with a monoclonal antibody, which itself is liable to form a complex with said peptide YSSPGSPGT or YSSPGSPGT.

3. Monoclonal antibody according to any one of claims 1 and 2,

- which is not liable to form an immunological complex with normal tau protein,
- which is not liable to form an immunological complex with tau protein present in brain homogenates derived from human brain, the homogenates being isolated from a patient having died of non-neurological disorders,
- which is not liable to form an immunological complex with the above-defined epitope previously treated with a dephosphorylating agent, such as alkaline phophatase,
- which is not liable to form an immunological complex with any variant peptide defined above and previously treated with a dephosphorylating agent, such as alkaline phosphatase.

4. Monoclonal antibodies according to any one of claims 1 to 3, which are

- characterized by the fact that they form an immunological complex with the abnormally phosphorylated forms of tau protein, present in homogenates of human brain obtained from a patient having died of Alzheimer's disease and
- characterized by the fact that these abnormally phosphorylated tau proteins present an apparent molecular weight which is higher than that of normal tau proteins, with said normal tau proteins being derived from brain homogenate, itself isolated from a patient having died of non-neurological disorders and
- characterized by the fact that, in these abnormally phosphorylated tau proteins, the apparent molecular weight can be decreased to that of normal tau proteins upon treatment of said abnormally phosphorylated tau proteins with a dephosphorylating agent.

5. Monoclonal antibody secreted by the hybridoma deposited at ECACC on October 08, 1991 under No. 91100806.

6. Hybridoma, which secretes a monoclonal antibody according to any one of claims 1 to 5.

7. Peptides which can be obtained from a brain homogenate, itself isolated from the cerebral cortex obtained from a patient having Alzheimer's disease and which forms an immunological complex with the monoclonal antibody according to anyone of claims 1 to 5.

8. Peptides liable to form an immunological complex with any of the monoclonal antibodies, according to the monoclonal antibodies of claims 1 to 5,
- which contain or are constituted by the sequence YSSPGSPGT or YSSPGSPGT, preferably YSSPGSPGT, phosphorylated at the positions marked with *, or
- which contain or are constituted by the sequence of the peptides liable to form an immunological complex with a monoclonal antibody according to any one of

claims 1 to 5, which itself is liable to form a complex with the peptide YS$\overset{*}{S}$PG$\overset{*}{S}$PGT or YSSPG$\overset{*}{S}$PGT.

9. Peptides of about 100 amino acids

- which contain the sequence YS$\overset{*}{S}$PG$\overset{*}{S}$PGT or YSSPG$\overset{*}{S}$PGT,preferably YSSPG$\overset{*}{S}$PGT, phosphorylated at the positions marked with *, or
- which contain the sequence of the peptides liable to form an immunological complex with a monoclonal antibody according to anyone of claims 1 to 5, which itself is liable to form a complex with the peptide YS$\overset{*}{S}$PG$\overset{*}{S}$PGT or YSSPG$\overset{*}{S}$PGT.

10. Peptides according to any one of claims 7 to 9, which are liable to generate a monoclonal antibody according to any one of claims 1 to 5.

11. Peptides which are contained in the brain, in the cerebrospinal fluid, or the serum of a patient having Alzheimer's disease or any brain disease involving PHF or tau protein and which forms an immunological complex with a monoclonal antibody according to any one of claims 1 to 5.

12. Peptides (antigens)

- which contain the sequence

$$(X_1)_p(X_2)_q\overset{*}{S}PX_3\overset{*}{S}P(X_4)_r(X_5)_s \text{ or}$$
$$(X_1)_p(X_2)_qSPX_3\overset{*}{S}P(X_4)_r(X_5)_s,$$
preferably $(X_1)_p(X_2)_qSPX_3\overset{*}{S}P(X_4)_r(X_5)_s$,

in which X_1, X_2, X_3, X_4, X_5 are any one of the 20 amino acids and p, q, r, s are 0 or 1, phosphorylated at places marked by *,

provided that said peptide is able to form an immunological complex with a monoclonal antibody according to any one of claims 1 to 5.

13. Abnormally phosphorylated tau protein characterized such as obtained from normal tau protein which is subjected to phosphorylation by any kinase which is capable of phosphorylation of normal tau at

the positions marked with * in the normal tau amino acid sequence YSSPGSPGT or YSSPGSPGT.

14. Process for obtaining and isolating a hybridoma secreting a monoclonal antibody according to any one of claims 1 to 5, characterized in that it involves:

- starting from the spleen cells of an animal, e.g. mouse or rat, previously immunized in vivo or from spleen cells of such cells previously immunized in vitro with an antigen recognized by the monoclonal antibody deposited at ECACC on October 8 under N° 91100806
- fusing said immunized cells with myeloma cells under hybridoma-forming conditions; and
- selecting those of the hybridomas which secrete the monoclonal antibodies which specifically recognize an epitope of the peptide of any one of claims 7 to 10 and which form an immunological complex with said epitope.

15. Process for producing monoclonal antibodies according to any one of claims 1 to 5 which involves:

- culturing the selected hybridomas according to claim 6, in an appropriate medium culture; and
- recovering the monoclonal antibodies excreted by said selected hybridomas;

or alternatively:

- implanting the selected hybridomas of claim 6 into the peritoneum of a mouse and, when ascites has been produced by the animal, recovering the monoclonal antibodies then formed from said ascites.

16. Process for the preparation of the peptide according to any one of claims 7 to 12, starting from said peptide in non-phosphorylated form which involves:

- reacting said peptide, which is non-phosphorylated, with a kinase enzyme capable of recognizing the non-phosphorylated epitope of the peptide and of modifying the epitope to a phosphorylated epitope recognized by the monoclonal antibodies according to anyone of claims 1 to 5.

17. Process for the detection or diagnosis in vitro of brain disease involving PHF and tau protein, e.g. Alzheimer's disease, which involves:
- contacting a monoclonal antibody according to any one of claims 1 to 5, with a preparation of NFT or a detergent-extracted brain homogenate isolated from a patient having had Alzheimer's disease under conditions suitable for producing an antigen-antibody complex; and
- separating the antigen from said complex and recovering the antigen sought in a purified form.

18. Process for the detection or diagnosis in vitro of brain disease involving PHF and tau protein, e.g. Alzheimer's disease, which includes:
- bringing a sample of brain homogenate, or of cerebrospinal fluid, or of serum from a patient suspected of suffering from a neurological disorder involving tau protein and PHF, more particularly Alzheimer's disease, into contact under in vitro conditions with a monoclonal antibody according to any one of claims 1 to 5, under conditions suitable for producing an antigen-antibody complex; and
- detecting the immunological binding of said antibody to said sample of brain homogenate, or of cerebrospinal fluid, or of serum.

19. Kit for the diagnosis in vitro of one of the following diseases: Alzheimer's disease, Down's syndrome, Pick's disease, SSPE and other neurological disorders in which abnormally phosphorylated tau protein or paired helical filaments are implicated, characterized in that the kit comprises:
- at least a microplate for deposition thereon of any monoclonal antibody according to any one of claims 1 to 5;
- a preparation containing the sample to be diagnosed in vitro, possibly together with a labeled peptide containing the epitope of the invention and preferably with the peptide YSSPGSPGT or YSSPGSPGT, preferably

YSSPG*S*PGT, phosphorylated at the positions marked with *.

- a second antibody

. which can be a monoclonal antibody recognizing an epitope of normal tau, or of abnormally phosphorylated tau protein, or of a peptide of any one of claims 7 to 12, with said epitope being different from the one of the invention, or

. which can be a polyclonal antibody of normal tau, or of abnormally phosphorylated tau or of a peptide of any one of claims 7 to 12, with said polyclonal antibody being liable to form an immunological complex with epitopes which are all different from the epitope of the invention, with said polyclonal antibody being preferably purified by immunoaffinity chromatography using immobilized tau protein, or

- a marker either for specific tagging or coupling with said second antibody;

- appropriate buffer solutions for carrying out the immunological reaction between the monoclonal antibody of the invention and a test sample on the one hand, and the bound second antibody and the marker on the other hand,

- possibly a peptide of any one of claims 7 to 12 for standard purposes, or for competition purposes with respect to the antigen which is sought.

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